EXTRACTION OF GLYCOLIC ACID FROM NATURAL SOURCES

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Report submitted in partial fulfillment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

JANUARY 2012

ABSTRACT

Glycolic acid (GA) is a type of alpha hydroxyacetic acid (AHA). It is a small molecule of AHA that is colorless, odorless and hygroscopic. Since GA is capable of penetrate the skin, it is suitable for exfoliate and anti ageing application. It is widely used especially in dermatology, medical and pharmaceutical applications. Since GA is very useful in cosmetic and pharmaceutical field, it yields a high market demand. By using rotten fruits obtained from the market as the sources to produce GA, the waste management in the market could be overcome. Thus, the production cost of GA could be reduced. The purpose of this study is to study the highest production of GA from different type natural sources such as sugarcane juice and banana in term of fresh and rotten state. The study is ought to investigate the effect of concentration of ethylene glycol (0.2 M to 1 M), temperature (40 ºC to 80 ºC) and time (30 min to 60 min) on extraction of GA compounds from natural sources and to optimize the production of glycolic acid from the sample after screening using response surface methodology (RSM). The ultrasonic homogenizer was used for the extraction of product with different independent variables. HPLC is used to analyze the concentration of GA in the samples. From the present research, fresh banana peels contain the highest GA concentration at 0.914 M. From RSM the most optimum combination variables are temperature at 70 ºC, solvent concentration at 1 M and extraction time at 50 min with desirability 1.000. The optimization of GA can be further investigated by using different types of solvent, extraction method and ultrasonic frequency.

ABSTRAK

Asid glikolik (GA) adalah sejenis asid alfa hidroksil asetik (AHA). Ia adalah molekul kecil AHA yang tidak mempunyai warna, bau dan bersifat higroskopik. Memandangkan GA mampu menembusi lapisan kulit, ia sesuai digunakan untuk pengelupasan dan anti penuaan. Ia digunakan secara meluas dalam bidang dermatologi, perubatan dan farmaseutikal. Disebabkan GA sangat berguna dalam bidang kosmetik dan farmaseutikal, ia mempunyai permintaan yang sangat tinggi. Dengan menggunakan buah-buahan yang rosak dari pasar sebagai sumber untuk menghasilkan GA, pengurusan bahan sisa di pasar dapat diatasi. Di samping itu kos penghasilan GA dapat dikurangkan. Tujuan kajian ini dijalankan adalah untuk mengkaji penghasilan GA yang paling tinggi daripada sumber semula jadi yang berbeza-beza, iaitu daripada air tebu dan pisang (*sebatu*) sama ada dalam keadaan segar atau rosak. Kajian ini dijalankan untuk mengkaji kesan kepekatan ethylene glikol (0.2 M hingga 1 M), suhu (40 ^{o}C hingga 80 ^{o}C) dan masa (30 min hingga 60 min) untuk pengekstrakan GA daripada sebatian sumber semula jadi dan untuk mengoptimumkan pengeluaran GA daripada sampel selepas menggunakan kaedah sambutan permukaan (RSM). Homogenizer ultrasonik digunakan untuk mengekstrak produk dengan pembolehubah bebas yang berbeza. Kromatografi cecair berprestasi tinggi (HPLC) digunakan untuk menganalisa kepekatan GA dalam setiap sampel. Keputusan kajian ini telah menunjukkan kulit pisang segar mengandungi kepekatan GA yang paling tinggi iaitu 0.914 M. Berdasarkan kombinasi pembolehubah yang ditentukan dari RSM, keadaan yang paling optimum adalah pada suhu 70 ^{o}C, kepekatan pelarut pada 1 M dan masa pengestrakkan 50 min dengan kecenderungan 1.000. Kajian pengoptimuman GA yang lebih lanjut boleh dilakukan dengan menggunakan jenis pelarut, kaedah pengekstrakan dan frekuensi utrasonik yang berbeza.

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LIST OF SYMBOLS

A_1	Fresh banana peel
A_2	Rotten banana peel
B_1	Fresh banana pulp
B_2	Rotten banana pulp
b_0	Constant term
b_1, b_2, b_3	Linear effects
b_{11}, b_{22}, b_{33}	Quadratic effects
b_{12}, b_{13}, b_{23}	Interaction effects
C_1	Fresh sugarcane
C_2	Rotten sugarcane
X_1	Temperature
X_2	Solvent concentration
X_3	Time
y	Response function

LIST OF ABBREVIATIONS

AHA	Alpha hydroxyl acid
ANOVA	Analysis of variance
CCD	Central composite design
EG	Ethylene glycol
GA	Glycolic acid
GC	Gas chromatography
HPLC	High performance liquid chromatography
IC	Ion chromatography
LC	Liquid chromatography
RSM	Response surface methodology
US$	American dollar
₩	Won (South Korea money name)
¥	Renminbi (China money name)

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Cosmetic industry has a high potential due to increasing consumers around the world. It was reported, in 2010 cosmetic industry in South Korea was expected to grow to ₩8 trillion (US$6.7 billion) by Amorepacific, Korea's biggest cosmetics company. According to Analysys International, China's online retailing market is aspected to increase to ¥713 billion (US$104.4 billion) from 3.56% of the nation's total social commodity retail sale in 2012.

Nowadays, environmental pollution generated from economics activities such as chemical, petrochemical, agricultural and food industries are common problems faced by the world. There is a potential for solid waste from fruits to be used as raw material, or for conversion into useful and higher value added products. The fruit waste can be used to produced protein, ethanol, methane, pectins, extracts and enzymes.

Glycolic acid (GA) is considered as a very important chemical compound with significant application in pharmaceutical, chemical industry and has been well known for being used as a cosmetic ingredient and a superficial peeling agent in dermatology (Kataoka et al., 2001). Recently, the use of glycolic acid containing cosmetics has received increasing public interest owing to their supposed ability to improve acne as well as premature aging of the skin (Clark et al. 1996 and Murad et al. 1995) to reduce wrinkles, roughness, age spots and other skin damage (Males and Herring, 1999). It can be prepared by chemical synthesis and be produced from fermentation broth or from glycolonitrile hydrolysis by mineral acid such as sulfuric acid (Grether and Vall, 1936; Shi, et al., 2005).

In plants, GA is an important intermediate in the photorespiratory carbon oxidation cycle (Jolivet et al., 1985). Experiment conducted by Jolivet et al. (1985), in order to examine the rate and sequence of photorespiratory metabolism following ^{18}O incorporation in the glycolate synthesized by leaves exposed to $^{18}O_2$. Under these conditions, the glycolate analyzed by mass spectrometry was labeled in ^{13}C and ^{18}O. The GC-MS analytical method which has been developed is suitable for the quantitative determination of GA especially from plant extracts. However in this research, HPLC been applied for GA analysis due to accuracy and better quantification.

1.2 PROBLEM STATEMENT

Usually banana can be found in the local market. Normally, bananas do not stay fresh for long. They are always being sold in a bunch at local markets and the flesh is commonly sold as banana fritters while the peel will be discarded and become a solid waste. However, if waste can be transformed into a valuable product such as organic acid, this would heighten the profits and competitiveness of the industry. For instance, the banana waste collected from the local market can be used as a substrate for organic acid production such as glycolic acid (GA). Therefore, the use of banana waste for glycolic acid production may be an option for utilizing low value waste material in producing a commercial product while solving environmental problems. Previous research use enzymatic methods to produce GA, however those method suffer from instability and high cost of the commercially purified enzyme glycolate oxidase (Oungpipat and Alexander, 1994). Hence reduce the price of glycolic acid.

In this study, glycolic acid been produced because it has a high market demand especially in the cosmetic and pharmaceutical field (Clark et al. 1996 and Murad et al. 1995). Based on Bergfeld et al. (1997) results illustrated that GA treatment is superior to mechanical exfoliation in improving the cosmetic appearance of photo aged skin. Zhu et al. (2004) and Chauhan et al. (2002) mentioned that it is also used in numerous areas of technology such as adhesive, metal cleaning and electroplating.

1.3 OBJECTIVE

The main objective of this research is to optimize the production of glycolic acid (GA) from natural source. The measureable objectives are:

a) To screen natural sources (sugarcane juice and banana) that contain high yield of glycolic acid.
b) To determine the effect concentration of solvent (ethylene glycol).
c) To determine the effect of temperature.
d) To determine the effect of extraction time.

1.4 SCOPE

Glycolic acid (GA) can be produced from plants extraction or chemical synthesis. In this study, GA will be produced from two natural sources from rotten and fresh fruits, which will be collected from the local market. Six types of samples that will be used are fresh banana peel, fresh banana pulp, fresh sugarcane, rotten banana peel, rotten banana pulp and rotten sugarcane. Sample that produces the highest production of glycolic acid will be chosen for optimization using response surface methodology (RSM). Variable parameters used are temperature in the range 40 ^oC to 80 ^oC, solvent concentration from 0.2 M to 1 M of ethylene glycol and extraction time in range 30 min to 60 min. Ultrasonic homogenizer be used for extraction of glycolic acid. The analysis of the glycolic acid will be done by using high performance liquid chromatography (HPLC).

2.4 RATIONALE AND SIGNIFICANT

This research, high production of glycolic acid isolated from natural sources has potential cosmetic and pharmaceutical benefits especially as a therapeutic skin corrector. It is a chemical exfoliant that can regenerate the skin (Kataoka et al., 2001). In fact, it can act as a useful adjuvant for the treatment of acne because of its unique properties (Chauhan et al. 2002 and Katherine, 2000). The significant of this research is to help waste management in the market by reducing solid waste from fruits. This could also help fruit's seller in Malaysia by buying solid waste from fruits rather than throwing them away. Economic effective because can produce GA by our own technology instead of importing from other country.

CHAPTER 2

LITERATURE REVIEW

2.1 ALPHA HYDROXY ACIDS

Alpha5hydroxyl acids (AHA) are water-soluble and are often added to skin care products. AHA mostly prepare by chemical synthesis or fermentation. AHA are also referred to as ‘fruit acids.’ It is usually found in citrus fruits, apricots, apples, grapes and sugarcane juice. AHA are meant to improve skin problems such as pimples, blackheads, whiteheads, reduce acne scars, improve skin’s texture and bring out radiance, treat fine lines, wrinkles and lighten freckles. AHA promotes the shedding of the dead cells and instigates the renewal scene to take place in order to make skin smooth-looking and radiant. AHA is usually used for medical and pharmaceutical application (Van Scott and Yu, 1974).

2.2 GLYCOLIC ACID

2.2.1 Glycolic Acid

For many year glycolic acids (GA) have been used in cosmetic products to remove undesirable signs of skin ageing (Kurtzweil, 1998). GA or defined by IUPAC as hydroxyethanoic acid is a type of fruit acids or alpha hydroxyl acid (AHA). Other names for GA are hydroxyacetic, glucohydroxyacid and kyselina glykolova.

According to Chauhan et al. (2002), GA is crystalline, colorless, odorless and hydroscopic. GA penetrates easily into the skin as compared to other types of alpha hydroxyl acid because it is the smallest molecule within the homologous series of AHA with two carbon atoms. It has high acidity but easily soluble in water and proved to be an effective dermatologic and cosmetic ingredient as it can be used as natural skin exfoliant and moisturizer (Katoaka et al., 2001). It is also easy soluble in methanol, ethanol, acetone, ethyl acetate, ether and acetic acid (Budavari, 1996 and Miltenberger, 1989)

GA is a low mole weight compound (76.05 g/mol) but has unique properties and dual functionality of alcohol and acid. In acidic condition, it forms cyclic and linear polymers known as glycolides. In aqueous environments GA dissociates into glycolate and hydrogen ion. Make GA ideal for a broad spectrum of consumer and industrial applications such as rust removal and degreasing (Chauhan et al., 2002).

Katoaka et al. (2001) stated that it is prepared by chemical synthesis or extraction from plants. According to Chauhan et al. (2002) GA can be produced from fermentation broth or from glycolonitrile hydrolysis by mineral acid such as sulfuric acid (Grether and Vall, 1936 and Shi et al. 2005).

2.2.2 Benefits of Glycolic Acid

There are a lot of benefits from GA such as stimulated the synthesis of new collagen (Van Scott and Yu, 1989) and decreasing keratinocytes cohesion (Katherine, 2000).

Researchers found that GA in low concentrations decreases corneocyte cohesion by promoting exfoliation of the outer layers of the stratum comeum (Katz, 1995 and Van Scott and Yu, 1984). This is important because most pigmentation alterations associated with photo damage can be attributed to be thickening of the stratum comeum (Bergfeld et al., 1997).

Ditre et al. (1996) noted that a significant increase in overall epidermal thickness that appeared to be secondary to augmented synthesis of glycosaminoglycans and collagen. Besides that they also found significant reversal of basal cell atypia, dispersal of melanin pigmentation, and a return to a normal pattern (Ditre et al., 1996).

GA can act as a useful adjuvant for the treatment of acne. The combination with topical retinoid makes it more effective in preventing comedonal acne (Katherine, 2000).

Stagnone (1989) reported that, repeated and regular applications of GA to the face have been shown to diminish fine facial wrinkles significantly.

2.2.3 Application of Glycolic Acid

Glycolic acid, perhaps the best-known AHA, is used in various fields. It is widely used especially in dermatology (Katherine, 2000), medical and pharmaceutical applications (Guzel, 1996; Moy et al. 1993; Van Scott and Yu, 1984 and Van Scott and Yu, 1989).

William (1978) reported that the concentration of glycolic acid in biological fluids has been used as an index for differential diagnosis of the hyperoxaluria. It is also used as inhibitors for harmful oxidation biochemical processes (Moy et al., 1993).

GA is one of the most important fine chemicals. It is used in numerous areas of technology such as in adhesive, metal cleaning, textiles, leather processing (Krochta et al., 1988), biodegradable polymers (Hayes and Lauren, 1994), electroplating, dairy cleaning, water-well cleaning, masonry, detergents (Kirk-Othmer, 1981), and as a component in personal care product (Yamamoto et al., 2006). Besides that, GA is also used as automotive oil additives, oil and water well flow enhancers, pH controlling, cosmetics, and chemical intermediate manufacture (Guzel, 1996 and Van Scott and Yu, 1984).

2.2.4 Glycolic Acid Structure

The structures of AHA family group are illustrated in Figure 2.1. GA permeates at slightly slower rate because it is more hydrophilic than acetic acid (Leo et al., 1971).

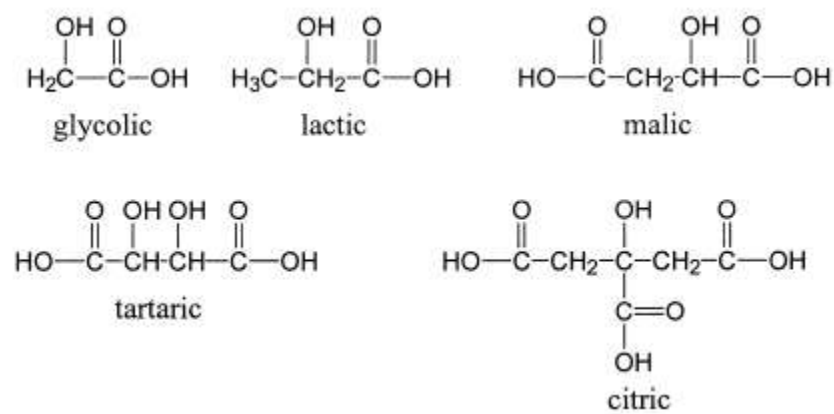

Figure 2.1: The alpha hydroxyl acid family

Adapted from: Males, R.G. and Herring, F.G. 1999. A H-NMR study of the permeation of glycolic acid through phospholipid membranes. *Biochimica et Biophysica Acta*. **1416**(1-2): 333-338

GA can diffuse easily throughout the intercellular phase in plasma and skin because it is hydrophilic and small compound of AHA. Smith (1994) proved that GA has a pH-dependent ability to stimulate cell renewal and it was observed at pH 3.

2.3 ANALYSIS

Numerous methodologies have been applied for the measurement of GA. These include calorimetric (Niederwieser et al. 1978 and Viccaro et al. 1972), isotope dilution (Hockaday et al., 1965), chromatographic (Petrarulo et al. 1989; Petrarulo et al. 1990 and Wandzilak et al. 1991) and enzymatic methods (Bais et al. 1985 and Kasidas and Rose, 1979).

Oungpipat et al. (1994) claimed that colorimetric, isotope dilution, gas chromatographic (GC), liquid chromatographic (LC) and enzymatic methods have inherent problems in analysis GA. The colorimetric methods are non-specific. The isotope dilution methods are used in combination with calorimetric methods, which are unreliable. GC and LC methods require complex isolation and derivatisation steps as well as involve the use of an expensive apparatus. Enzymatic methods suffer from instability and high cost of the commercially purified enzyme glycolate oxidase (Oungpipat et al., 1994).

GA are examined using chromatographic methods, including gas chromatography (GC) (Asano, 2002) after derivatization, ion chromatography (IC) (Chen et al., 2005) and liquid chromatography (HPLC) (Scalia et al., 1998). De Bruijn et al. (1984) concluded that even though GC provided superior resolution, HPLC had advantages of shorter sample preparation and analysis times.

Other researchers proved that HPLC is the best method for the analysis of GA and other acid compounds in sugar (Abeydeera, 1983; Morel du Boil et al. 1990; Thompson et al. 1990; Tsang et al., 1990 and Schäffler et al. 1990).

2.4 SUBSTRATE

GA is mainly extracted from sugarcane, sugar beets and various fruits (Chauhan et al., 2002). Van der Poel et al. (1998) proved that cane juice contains GA and other organic acid such as citric, malic, succinic, fumaric, lactic, oxalic, acetic, formic, itaconic and aconitic acid (1-propene-1,2,3-tri-carboxylic acid).

Table 2.1: Glycolic acid from various sources

Source	Authors
Tomato (*Lycopersicum esculentum* Mill.) Maize leaves (Zea mays L.)	Jolivet et al. (1985)
Algae	Tolbert and Zill (1956) Fogg (1966) Watt (1969) Jolivet et al. (1985)
Glycolonitrile hydrolysate	Yunhai et al., (2006)
An aqueous solution of glyoxal in the presence of a catalytic	Kiyoura and Kogure (1997)
Ethylene glycol	Wei et al. (2009)
DGA-utilizing *Rhodococcus* sp. No. 432	Yamanaka et al. (1991)
Paenibacillus sp. AUI 311	Isobe et al. (2007)
Gluconobacter oxydans DSM 2003	Wei et al. (2009)
Microorganisms	Kataoka et al. (2001)

2.4.1 Banana

Bananas are a tropical fruit from *Musaceae* family and produced in large quantities. In Malaysia, it was estimated to be 33,704.2 hectares total planted area of bananas in 2001 (MAO, 2006). Banana peel contains 30% to 40% of total banana weight (Oberoi et al., 2011). Nowadays, there is a large quantity of banana peel was wasted and consumed as animal feed. This activity is unfriendly to the environment and non-economical. Based on the reasons, banana-processing industries have been searching for applications of these by-products which proved to be a source of important natural compounds, such as protein, ethanol, methane and pectin (Cordenunsi et al. 2008 and Willats et al. 2001). Banana peel also contains highly valued antioxidant compounds and can be used as food livestock and biosorbent for heavy metals, dyes and the removal of phenolic compounds (Achak et al. 2009; Annadurai et al. 2002; Anwar et al. 2010 and Onwuka et al. 1997).

According to Juliana et al. (1989), raw banana juices are turbid, gray in colour, very viscous and tend to settle during storage and contain polysaccharides such as pectin and starch. Research doing by reported that green banana flour contained high total starch, resistance starch and dietary fibre content about 73.4%, 17.5% and ~14.5% and high-sugar content in ripe banana.

2.4.2 Sugarcane

Brazil and Australia are the biggest county planted sugarcane (*Saccharum Officinarum*). In sugarcane, organic acid constitutes a significant proportion of the ionic organic non-sugar. Sugarcane contains organic acid such as citric, malic, succinic, fumaric, glycolic, lactic, oxalic, acetic and aconitic acic (Walford, 2002). Solid waste from sugarcane like bagasses can be applied for animal feed, paper, pulp and board (Banerjee and Pandey, 2002).

2.5 SOLVENT

Acid can be used as catalysts for fruit's hydrolysis because according to Aguilar et. al. (2002), acid can break down heterocyclic ether bonds between sugar monomers in the polymeric chain, which are formed by hemicellulose and cellulose.

Ethylene glycol is one of the cheap starting materials, can be use for the production of glycolic acid through an oxidation reaction (Katoaka et al., 2001). It was expected that microbial conversion of ethylene glycol to glycolic acid was an attractive alternative method for the value-added production of glycolic acid with no by-production. (Wei et al., 2009).

CHAPTER 3

METHODOLOGY

3.1 RESEARCH DESIGN

Figure 3.1 simplified the process methodology of GA production.

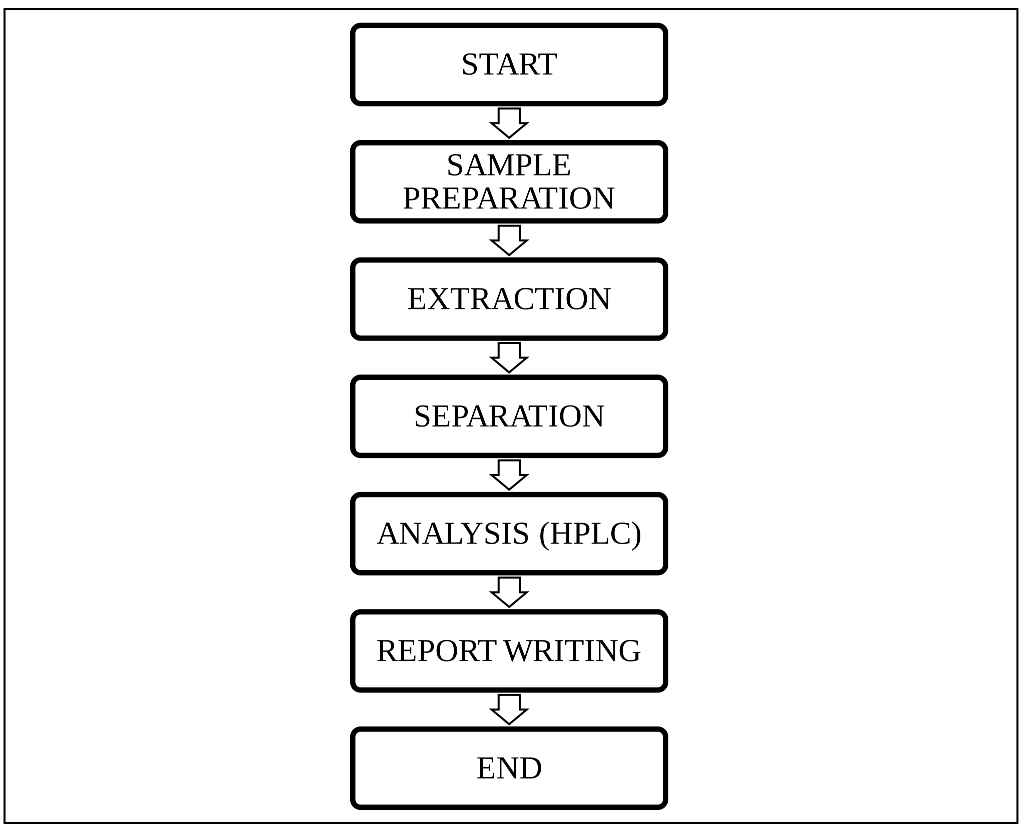

Figure 3.1: Process methodology of GA production

3.2 PROCEDURE

3.2.1 Reagents

All the chemicals used were of analytical grade and purchased from various suppliers.

3.2.2 Natural Sources

Banana (*pisang sebatu*) and sugarcane juice (Figure 3.2) are obtained from local market. Half of the fresh banana and sugarcane juice were kept for a few days to prepare rotten fruit samples.

Figure 3.2: Fresh banana (*pisang sebatu*) and sugarcane juice

3.2.3 Sample Preparation

The fresh and rotten fruits were washed and separated into pulp and peel (Figure 3.3). Banana pulp and peel were cut into small pieces and dry into the oven (MEMMERT, Model CELSIUS 2000, Malaysia) at 60 ^{o}C for several days.

Figure 3.3: Separation of banana peel and pulp

The dried peel and pulp were blended with blender (SHARP, Model EM-11, Malaysia). The fresh and rotten sugarcane juices were freeze dried (BioTron, Model Clean vac 8) for several days.